Original Article

Roles of intrinsic and acquired resistance determinants in multidrug-resistant clinical *Pseudomonas aeruginosa* in Bangladesh

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Abstract

Introduction: *Pseudomonas aeruginosa* is an ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa, and Enterobacter spp*.) pathogen and one of the leading etiologies in multiple nosocomial infections. Treatment of *P. aeruginosa* is becoming increasingly difficult due to its ever-increasing antibiotic resistance trends. This study investigated clinical multidrug resistance (MDR) *P. aeruginosa* (MDR-PA), their intrinsic resistance determinants, including the presence of chromosomal AmpC β-lactamase (Ampicillinase), decreased expression of outer membrane porin protein OprD and selected acquired β-lactamase resistance genes. ***Methods:*** Out of 238 clinical specimens, including urines from urinary tract-infected patients, wound swabs, burn swabs, and catheter aspirates, were collected from two major hospitals in Savar, Dhaka, Bangladesh. Samples were inoculated with Cetrimide agar to isolate presumptive *P. aeruginosa*. Bacteria were identified by cultural, biochemical characterization, 16S rDNA sequencing, and phylogenetic analysis. Virulence-associated genes of *P. aeruginosa*, namely, *toxA*, *lasB*, and *plcH*, were identified by polymerase chain reaction (PCR). Antibiotic susceptibilities of the isolates were investigated against ten antibiotics belonging to seven groups by disc-diffusion method followed by a selected minimum inhibitory concentration (MIC) assay. Phenotypic expression of Metallo-β-lactamases (MBLs) production was checked by the double disc synergistic test selectively among the imipenem-resistant isolates. Acquisition of β-lactam resistance trait was examined by PCR detection of *bla*-genes variants. Mutational loss of the OprD was analyzed by PCR to investigate intrinsic resistance determinants. Phenotypic overexpression of chromosomal AmpC was assayed with the identification of the AmpC gene by PCR. The expression level of OprD was assessed by real-time quantitative PCR (RT-qPCR). ***Results:*** Fifty-three P. *aeruginosa* was identified, with an overall isolation of 22.3% (53/238), where urine remains the most prevalent source. Virulence genes *toxA*, *lasB*, and *plcH* were identified in the isolates of 92.4%, 96.2%, and 94.3%. The highest phenotypic antimicrobial resistance was observed against ampicillin and ceftriaxone (100%), followed by cefotaxime (96%), tetracycline (89%), azithromycin (72%), imipenem (31%), ciprofloxacin (29%), levofloxacin (29%), gentamycin (27%) and ceftazidime (14%). The antibiogram pattern revealed 85% of isolates as multidrug-resistant, while 12% were considered extensively drug-resistant (XDR)-*P. aeruginosa*. The carriage of β-lactamase genes bla_{TEM}, bla_{SHV}, and bla_{OXA} was detected in 4%, 2%, and 2% cephalosporin-resistant isolates, respectively. Double disc synergistic test revealed 87% of imipenem-resistant isolates expressing MBL-mediated resistance phenomenon. All seven ceftazidime-resistant isolates showed the presence of the AmpC gene with phenotypic overproduction of the AmpC enzyme, indicating AmpC-mediated ceftazidime resistance. Mutational loss of OprD was observed in 12% of phenotypically multidrug-resistant isolates, and RT-qPCR analysis revealed reduced expression of OprD porin protein at various levels in the outer membrane of multidrug-resistant isolates. ***Conclusions:*** This study depicts the high prevalence of MDR-PA in clinical specimens in Bangladesh. The identified intrinsic and acquired antimicrobial resistance determinants play synergistic roles in emerging MDR-PA.

Keywords: *Pseudomonas aeruginosa*, ESKAPE, OprD, Innate and Attained Resistance, Multidrug-resistant, Clinical Outcome, Bangladesh

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Introduction

Antibiotic resistance is one of the major concerns for treating bacterial infections. Multidrug-resistant (MDR) bacterial infections among hospitalized patients pose a significant challenge, resulting in a high mortality rate [1]. The global priority list of antibiotic-resistant bacteria labels *P. aeruginosa* as a critical pathogen requiring an in-depth study of their MDR nature [2]. *P. aeruginosa* is aerobic, non-fermenting, gram-negative bacilli commonly associated with severe nosocomial infections, including cystic fibrosis, secondary wound infections, ventilator-associated and hospital-acquired pneumonia, catheter-associated infections, burn wound infections, and urinary tract infections (UTIs) (Figure 1)[3, 4]. The bacteria are notorious for their rapid development of resistance against a wide range of therapeutics. Although empirical antibiotic therapy, including monotherapy and combination therapy, is generally effective [5, 6], recent trends show a steep increase in MDR, including resistance to β-lactams, quinolones, and aminoglycosides [7, 8].

β-lactam antibiotics, including cephalosporins, carbapenems, and monobactams, carry broad-spectrum antimicrobial activity and less toxicity and are considered relatively safe and effective options for treating infections caused by Gram-negative bacteria like *P. aeruginosa* [9, 10]. However, *P. aeruginosa* possesses many intrinsic and acquired resistance mechanisms against various antimicrobials [11]. Intrinsic resistance determinants include minimal outer membrane permeability, chromosomal AmpC β-lactamase (Ampicillinase) presence, and active broad-spectrum efflux pumps [12, 13]. The outer membrane permeability of *P. aeruginosa* is highly restricted; it is about 12-100-fold lower than *E. coli* [14, 15] (Figure 2). This is due to porin proteins like OprD, a highly mutable porin-containing binding site for several antibiotics, including carbapenems [16-18]. Loss or decreased expression of OprD is closely associated with the emergence of MDR in *P. aeruginosa* [16, 19]. Another important intrinsic β-lactam resistance mechanism is the derepression of

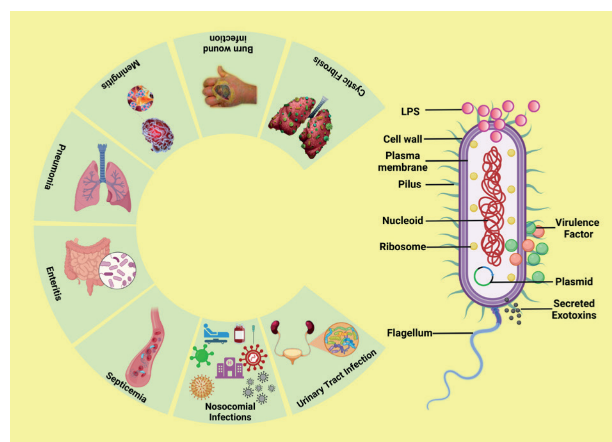

Figure 1: Schematic diagram showing different infections caused by *P. aeruginosa*. This figure has been drawn utilizing the premium version of BioRender with the License number ME2569JDYG. **Image Credit**: Susmita Sinha.

chromosomally encoded class C extended spectrum Ampicillinase (ESAC) like AmpC [20, 21]. Derepression and overproduction of AmpC in *P. aeruginosa* are associated with inhibiting antipseudomonal Cephalosporins like ceftazidime [22, 23]. *P. aeruginosa* also harbors broad-spectrum efflux pumps associated with resistance development, including MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexEF-OprN belonging to the RND (Resistance-Nodulation-Cell Division) superfamily [24, 25]. Overexpression of these efflux pumps plays a significant role in developing resistance against antibiotics like β-lactams, quinolones, and aminoglycosides [26, 27].

In contrast, the acquired resistance is mediated by the acquisition of resistance elements [28-30] like variants of extended-spectrum β-lactamases (ESBLs) [31] and MBLs through horizontal gene transfer and mutational overexpression of efflux pumps and Ampicillinase (AmpC) [32-34]. *P. aeruginosa* is shown to harbor a wide range of β-lactamases, complicating the treatment process [23]. ESBL harbored by *P. aeruginosa* includes class A β-lactamases like bla_{TEM} and bla_{PER-1} [35]; class B MBLs like bla_{IMP} and bla_{NDM} [36, 37]; class C chromosomal β-lactamase bla_{AmpC} [38, 39] and class D

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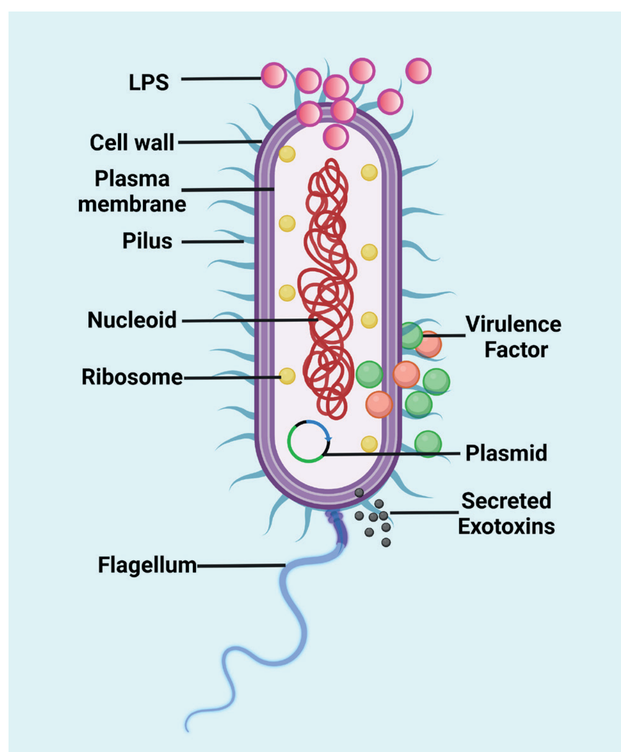

Figure 2: Schematic diagram showing the structure of the *Pseudomonas aeruginosa.* This figure has been drawn utilizing the premium version of BioRender with the License number OY2569KT5L. **Image Credit**: Susmita Sinha.

β-lactamase bla_{OXA} [40, 41]. Since the bacteria exhibit such an impressive arsenal of antibiotic-resistance elements [42, 43], *P. aeruginosa* has been considered a member of the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa,* and *Enterobacter*) group of pathogens [44] that encompasses the major MDR bacteria capable of get away the biocidal activities of antimicrobials [45-48].

P. aeruginosa has been explored in the context of Bangladesh, and its presence in healthcare facilities has been increasing alarmingly [49, 50]. It requires an in-depth study to investigate the occurrence of both acquired and intrinsic resistance determinants to understand the current situation of multidrug-resistant *P. aeruginosa* (MDR-PA) in healthcare facilities in Bangladesh. The current study investigates the role of different resistance mechanisms against β-lactam antibiotics in clinical *P. aeruginosa*, including acquired resistance determinants like ESBLs and MBLs, and intrinsic mechanisms like overproduction of AmpC and loss or decreased expression of OprD.

Materials and Methods

Sample Collection

This study collected clinical samples from patients admitted to Enam Medical College and Hospital; and Gonoshasthaya Samaj Vittik Medical College and Hospital located in the Savar area, Dhaka, Bangladesh, from 2019 through 2020. A total of 238 samples consisting of 115 midstream urines (48.4%), 69 pus (29%), 21 secondary wound infection swabs (8.9%), 12 urinary catheter swabs (5%), 10 burn wounds (4.2%), 9 blood (3.7%) and 2 tracheal aspirates (1%) specimens were investigated for the detection of *P. aeruginosa*. The samples were processed as per standard microbiological procedures. Data on the patient's clinical conditions and any prior use of antibiotics were collected alongside a validated structured questionnaire.

Bacterial Isolation and Identification

To identify *P. aeruginosa*, colony morphology, pigmentation, and fluorescence production were determined on Cetrimide agar (Scharlab SL, Spain). Isolates that can produce green pigmentation and fluorescence were selected for this study. According to Bergey's Manual of Systemic Bacteriology, they were subjected to conventional biochemical tests for presumptive indentification of *P. aeruginosa* [51-53]. Selected isolates were preserved in trypticase soya broth (TSB) with 20% glycerol at -30°C.

To confirm the identity, some selected isolates were subjected to a polymerase chain reaction (PCR) to amplify 16S rDNA as described elsewhere [54, 55]. The PCR products were purified and sequenced. Sequences were blasted with the online database of the NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm the identity of the bacterial isolates.

PCR Detection of Virulence Genes, ESBLs, and OprD

To determine the virulent nature of clinical *P. aeruginosa*, isolates were subjected to PCR detection for three virulence genes *toxA*, *lasB*, and *plcH*, encoding exotoxin A, elastase B, and phospholipase C, respectively. The presence of genes encoding ESBLs from Ambler Class A β-lactamase bla_{TEM}, bla_{SHV}, bla_{PER1}, Class C β-lactamase AmpC, and Class D β-lactamase bla_{OXA} were investigated in all isolates exhibiting phenotypic resistance to any β-lactam antibiotics explored in this study. Loss of OprD was detected by PCR amplification of the OprD gene. The primers and annealing temperatures used in this

study are enlisted in Table 1. For each PCR reaction, 2.0µl of prepared bacterial DNA was added to 8 µl of prepared PCR ready mix containing 5X buffer, 25mM $MgCl_2$, 1mM dNTP, deionized water, Taq DNA polymerase enzyme, and 10pmol of each primer (2 µl). PCR products were visualized using a UV gel documentation system after electrophoresis in 1% agarose gel.

Screening of Antimicrobial Resistance Phenomenon of P. aeruginosa

A bacterial susceptibility test to antibiotics was performed by disc-diffusion method [56, 57], followed by the determination of MIC by agar dilution method and interpreted according to the clinical laboratory standard institution (CLSI) guidelines [58]. Each *P. aeruginosa* isolate was checked for its exhibition of resistance phenotype against ten commonly used antibiotics belonging to seven clinically essential groups. The groups of antibiotics included penicillin (Ampicillin, AMP 10µg); cephalosporins (ceftriaxone, CRO 30µg; cefotaxime, CTX 30µg; ceftazidime, CAZ 30µg); carbapenem (imipenem, IMP 10µg); fluoroquinolone (ciprofloxacin, CIP 5µg; levofloxacin, LEV 5µg); aminoglycoside (gentamycin, CN 10µg); tetracycline (tetracycline, TET 30µg); macrolide (azithromycin, AZM 15µg). Antibiotic discs were obtained from Oxoid, UK. As control for antibiogram, *P. aeruginosa* ATCC 27853 was used.

Resistance phenotypes were considered when MDR-PA when the isolate showed resistance against ≥3 antimicrobial groups; isolates showing non-susceptibility to at least one agent in all but two or fewer antimicrobial groups were considered as extensively-drug resistant *P. aeruginosa* (XDR-PA) [59].

Phenotypic Detection of MBL-Production Using IMP-EDTA Disc Diffusion Method

IMP-EDTA double disc synergistic test was performed to detect of Metallo-β-lactamase (MBL) production. Sixteen Imipenem-resistant *P. aeruginosa* isolates were selected for this experiment to analyze the association between MBL production and carbapenem resistance. To prepare a bacterial lawn, pure culture of selected isolates was plated on Mueller Hinton agar (Oxoid, UK) using a sterile cotton swab. Two imipenem (IMP_{10}) discs were placed on bacterial lawns at a distance of at least 20mm. One of the imipenem discs was inoculated with 0.5M EDTA to achieve 750µg concentration. EDTA acts on Metallo-β-lactamase by removing zinc ions from the enzyme active site, conferring susceptibility to carbapenems. The plates were incubated at 37°C for 18 hours. Zone diameter was measured for the imipenem-EDTA disc and the imipenem disc alone. Isolates exhibiting ≥17mm inhibition zones with IMP-EDTA disc were considered MBL-positive, while isolates with ≤14mm inhibition zones were considered MBL-negative [60].

Phenotypic Detection of AmpC *B-Lactamase Overproduction*

The AmpC disc test conducted phenotypic detection of the overproduction of AmpC. Seven ceftazidime-resistant *P. aeruginosa* isolates were selected for the test to analyze the association between AmpC overproduction and resistance to ceftazidime. A sterile disc inoculated with an overnight culture of test isolate was placed next to a cefoxitin disc on a lawn of *E. coli* ATCC 25922 and incubated overnight. Following incubation, indentation or flattening of zone diameter was observed. Indentation of the zone of inhibition indicated a strong hyperproduction of AmpC, while flattening the zone was interpreted as weak AmpC production. An undistorted zone of inhibition was interpreted as a negative result [61].

Real-Time RT-PCR Analysis of OprD *Expression*

One-step real-time quantitative reverse-transcription PCR (RT-qPCR) was performed to quantify the level of expression of OprD. The expression levels were standardized according to the transcription level of the *rps*L ribosomal gene, which is expressed constitutively. Thirteen representative isolates were selected from different resistance profiles, and *P. aeruginosa* PAO1 was used as a reference strain in this experiment. OprD-negative isolates from PCR amplification were excluded. The quantification of mRNA present in the template was conducted using a NanoDrop™ spectrophotometer. One-step real-time quantitative PCR was performed using GoTaq® 1-step RT-qPCR System (Promega, USA) in a 20 µl reaction volume. The relative expression of OprD was compared to its expression on the PAO1 reference strain, and reduced expression relative to PAO1 (which was assigned a value of 1.0) was considered when the transcriptional levels of the isolates were ≤70% [62,63].

Statistical Analysis

A validated questionnaire was used for data collection. Collected data were verified and entered in IBM SPSS Statistics Data Editor (Version 21) and STATA 15 for subsequent analysis, and the figure was prepared by GraphPrism (Version 8.3.2). A p-value of <0.05

was considered significant. Logistic regression was used to estimate the odds ratio (OR) of specimens to resistance to different antibiotics and to become multidrug resistant in other specimens compared to urine specimens.

Ethical Approval

This study was approved by the Ethics and Research Review Committee of the Jahangirnagar University Faculty of Biological Sciences, Dhaka, Bangladesh [Ref No: BBEC, JU/M 2020 (1)4, Dated January 15-2020]. The research was conducted in collaboration with two private hospitals in Savar, Dhaka, Bangladesh, Gonoshasthaya Samaj Vittik Medical College Hospital and Enam Medical College Hospital. Both hospitals had accepted ethical permission from Jahangirnagar University, and the study was completed under formal ethical approval. Written informed consent was obtained from participants for sample collection, and their identities and other information were anonymized to protect their identities.

Results

Isolation and Identification of P. aeruginosa

Of 238 clinical samples, 53 appeared presumptive *P. aeruginosa* on selective cetrimide agar where fluorescent green pigmented colonies were noticed. The combined isolation was 22.3% (53/238), where secondary wound swabs remained the most prevalent source, with 52% isolation, followed by urinary tract catheters, which gave 42% isolation (Figure 3). Growth on MacConkey agar showed the cultures are non-lactose fermenters. Biochemical characterization revealed all 53 isolates positive for oxidase, catalase, nitrate reductase, and citrate utilization test while negative for lactose fermentation, indole, Methyl-Red, Voges-Proskauer tests, and hydrogen sulfide formation. 16S rDNA sequence analysis of our isolates showed 95% to 99% homology of different *P. aeruginosa* in the NCBI database.

Different clinical specimens include 115 midstream urines, 69 skin pus swabs, 21 secondary wound swabs, 12 urinary catheter swabs, and 21 other specimens, including burn wound swabs, blood samples, and tracheal aspirates were cultured on cetrimide agar. Pigmented fluorescence colonies were selected. Bacteria were identified by biochemical followed by 16s rDNA sequencing.

Detection of Selected Virulence Genes

PCR analyses identified the exotoxin A gene, *tox*A, in 49 (92.4%) *P. aeruginosa* isolates. The elastase B-encoding *las*B gene was detected in 51 (96.2%) isolates, and the phospholipase C-encoding gene, *plc*H, was detected in 50 (94.3%) isolates. About 93% of the isolates revealed carrying all three virulence factors. Only one isolate was found to have no *tox*A, *las*B, or *plc*H (Figure 4).

Table 1: Primers Were Used in This Study.

Primer	Sequence (5'-3')	Amplicon size (bp)	Annealing Temperature (°C)	Reference
*tox*A	F- GGA GCG CAA CTA TCC CAC T R- TGG TAG CCG ACG AAC ACA TA	150	50	[52]
*las*B	F- TTC TAC CCG AAG GAC TGA TAC R- AAC ACC CAT GAT CGC AAC	153	55	[52]
*plc*H	F- GAA GCC ATG GGC TAC TTC AA R- AGA GTG ACG AGG AGC GGT AG	307	55	[52]
bla_{TEM}	F- GAG TAT TCA ACA TTT TCG T R- ACC AAT GCT TAA TCA GTG A	857	50	[54]
bla_{SHV}	F- TCG CCT GTG TAT TAT CTC CC R- CGC AGA TAA ATC ACC ACA ATG	768	50	[54]
bla_{OXA}	F- GCA GCG CCG TGC ATC AAC R- CCG CAT CAA ATG CCA TAA GTG	198	50	[54]
bla_{PER1}	F-ATG AAT GTC ATT ATA AAA GCT R-TTA ATT TGG GCT TAG GG	927	45	[56]
OprD	F- CGC CGA CAA GAA GAA CTA GC R- GTC GAT TAC AGG ATC GAC AG	1413	61	[59]
AmpC	F- CTT CCA CAC TGC TGT TCG CC R- TTG GCC AGG ATC ACC AGT CC	1063	62	[59]
OprD (RT-PCR)	F- GCT CGA CCT CGA GGC AGG CCA R- CCA GCG ATT GGT CGG ATG CCA	Used for Quantitative RT-PCR		[59]
*rps*L	F- GCT GCA AAA CTG CCC GCA ACG R- ACC CGA GGT GTC CAG CGA ACC	Used for Quantitative RT-PCR		[59]

Polymerase chain reaction (PCR) was employed to amplify the listed genes. PCR products underwent

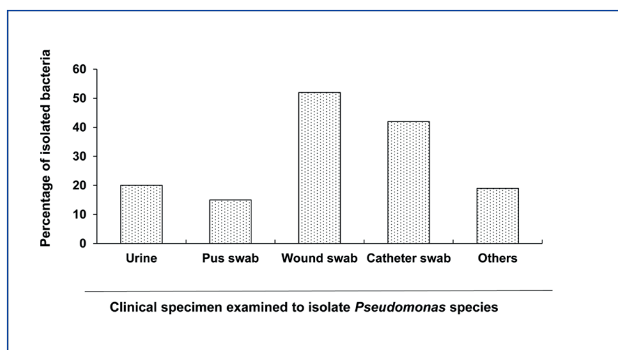

Figure 3: Relative abundance of *Pseudomonas* species in a different clinical specimen.

electrophoresis through 1% agarose gel, stained with ethidium bromide, and visualized under UV light. The 1 kb and 100bp DNA molecular markers (Promega Corporation, WI) were run alongside to estimate the size of the amplified PCR products. **A).** Amplified PCR products of virulence genes *toxA* (Exotoxin A), *lasB* (Elastase B), and *plcH* (Phospholipase C) were shown with molecular weights 150 bp, 153 bp, and 307 bp, respectively. **B.)** Detected ESBL genes bla_{TEM}, bla_{SHV}, and bla_{OXA} with molecular weights 857 bp, 768 bp, and 198 bp, respectively, were shown. **C).** The outer membrane porin protein gene (OprD, 1413 bp) was identified in most of the isolates. **D.)** The gene for class C extended spectrum ampicillinase (**AmpC***,* 1063 bp*)* was detected.

Antibiotic Susceptibility of the Clinical P. Aeruginosa Isolates

Antibiotic susceptibility test by the disc diffusion assay and minimum inhibitory concentration measurement revealed all isolates resistant to ampicillin and ceftriaxone (53/53). Isolates also showed high resistance against cefotaxime (96.2%), azithromycin (71.7%), and tetracycline (88.7%). The lowest resistance was observed against ceftazidime (13.2%, 7/ 53). The isolates exhibited a moderate level of susceptibilities to imipenem, ciprofloxacin, levofloxacin, and gentamycin (Figure 5).

The tested 53 *P. aeruginosa* demonstrated heterogeneous 14 different antibiograms profiles (RP01 to RP14) shown in Table 2. Almost three-fourths (73%, 39/53) of isolates exhibited resistance against three or more groups of antibiotics and became MDR. Four isolates belonging to RP14 showed resistance to all the antibiotics tested, while two isolates under RP13 became resistant to 9 antibiotics. Combining RP13 and RP14, six isolates (12%, 6/53) comprise extensively-drug-resistant *P. aeruginosa* or XDR-PA (Table 2). RP3 remained the single most predominant resistant profile of the isolates (45%,24/53), showing phenotypic resistance to β-lactams (ampicillin, ceftriaxone, cefotaxime), a macrolide (azithromycin) and tetracycline (Table 2).

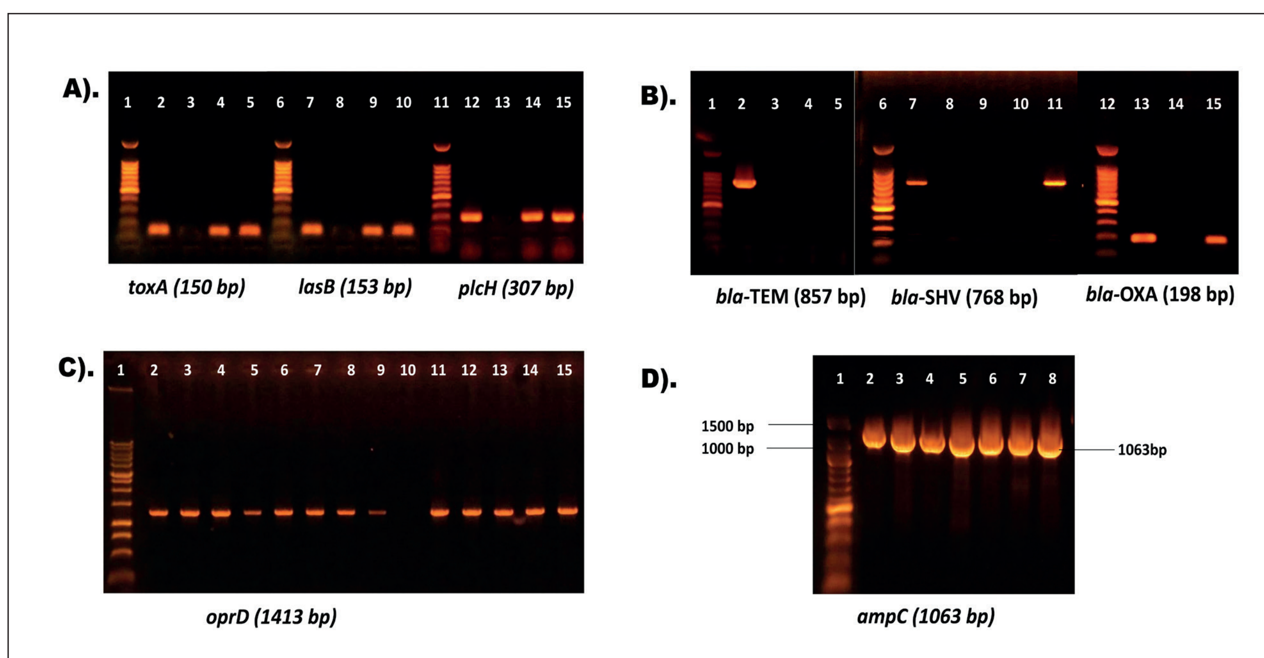

Figure 4: Detection of virulence genes, extended-spectrum **β**-lactamase (ESBL) genes, and the outer membrane porin protein gene (OprD).

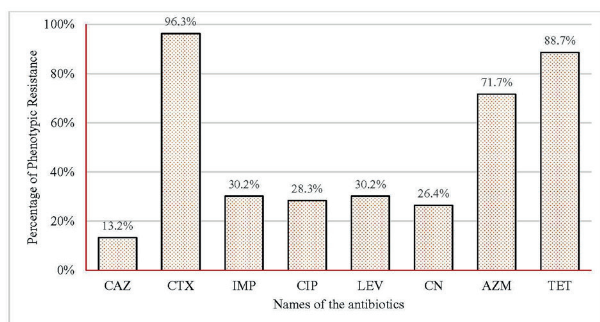

Figure 5: Graphical representation of resistance percentages of *P. aeruginosa* against different antibiotics. Notes: Isolates were subjected to minimum inhibitory concentration (MIC) assay by agar dilution. The susceptibility status of the bacteria was determined according to the CLSI interpretive criteria. Both AMP and CRO showed 100% resistance, thus not shown here.

Table 2: Antibiogram profiles of clinical *P. aeruginosa* isolates.

Profile	Resistance Phenotype	Number and (%) of the isolates
RP1	AMP-CRO	1 (1.9)
RP2	AMP-CRO-CTX-TET	7 (13.2)
RP3	AMP-CRO-CTX-AZM-TET	24 (45.2)
RP4	AMP-CRO-CTX-IPM-AZM	3 (5.7)
RP5	AMP-CRO-CTX-CAZ-AZM-TET	1 (1.9)
RP6	AMP-CRO-CTX-CIP-LEV-AZM	1 (1.9)
RP7	AMP-CRO-CTX-CIP-LEV-TET	1 (1.9)
RP8	AMP-CRO-CTX-IPM-CN-AZM-TET	2 (3.8)
RP9	AMP-CRO-CTX-CIP-LEV-AZM-TET	1 (1.9)
RP10	AMP-CRO-CTX-IPM-CIP-LEV-CN-AZM	1 (1.9)
RP11	AMP-CRO-CTX-CIP-LEV-CN-AZM-TET	1 (1.9)
RP12	AMP-CRO-CTX-IPM-CIP-LEV-CN-TET	4 (7.5)
RP13	AMP-CRO-CTX-CAZ-IPM-CIP-LEV-CN-TET	2 (3.8)
RP14	AMP-CRO-CTX-CAZ-IPM-CIP-LEV-AZM-TET-CN	4 (7.5)
Total		53 (100)

Notes: Here, %=percentage, AMP=Ampicillin; CRO=Ceftriaxone; CTX=Cefotaxime; CAZ=Ceftazidime; IPM= Imipenem; CIP= Ciprofloxacin; LEV= Levofloxacin; CN= Gentamycin; TET= Tetracycline; AZM= Azithromycin.

Detection of ESBL Genes

Isolates were subjected to PCR detection for the four variants of *bla* genes, namely bla_{TEM}, bla_{SHV}, bla_{OXA}, and bla_{PER1}. PCR results revealed that only 2 of 53 (3.8%) isolates carried bla_{TEM}. One of them also harbored bla_{OXA}, while the bla_{SHV} variant was present exclusively in one individual isolate (1/53; 1.9%). None of the isolates had the bla_{PER1} gene. Altogether, only 5.66 % (3/53) resistant isolates were found to carry the ESBL genes.

Metallo-β-Lactamase-Mediated Carbapenem Resistance

Among 16 imipenem-resistant isolates, 56% showed a ≥17mm zone of inhibition around the IMP-EDTA disk. About 44% (7/16) were found to produce a 15-16mm zone, and a single isolate did not make any zone of inhibition against the IMP-EDTA disk. Altogether, over 93% (15/16) phenotypic carbapenem-resistant *P. aeruginosa* isolates showed a confirmatory MBL-mediated carbapenem resistance phenomenon (Table 3).

Ceftazidime-Resistance Association with AmpC *Gene and Overproduction of* AmpC

Seven isolates were ceftazidime-resistant; all carried the AmpC gene detected by PCR. Phenotypic detection of AmpC overproduction was revealed in 6 of the 7 ceftazidime-resistant isolates (Table 4). The harmony of phenotypic overproduction of AmpC with its genotypic harborage was demonstrated well. None of the seven ceftazidime-resistant isolates carried bla_{TEM}, bla_{OXA}, or bla_{PER1} genes, whereas bla_{SHV} was detected in a single isolate (Table 4). The loss of OprD was observed in only one ceftazidime-resistant isolate (Table 4).

Association between Loss of OprD *and Multidrug Resistance*

All 53 isolates were evaluated for detecting the OprD gene by PCR. Six isolates showed negative results for OprD gene amplification, indicating loss of OprD porin protein. The association between the loss of OprD and MDR phenomena was observed: all OprD-negative isolates were found to be MDR-PA. We also noticed the loss of OprD with higher resistance to imipenem: imipenem resistance was detected in 66.7% (4/6) of the OprD-negative isolates compared to about 25% (12/47) of the OprD-positive isolates (p=0.06).

qPCR Analysis Reveals Reduced OprD *Gene Expression in MDR-PA*

For this experiment, fourteen isolates from different antibiotic resistance patterns were selected and

Table 3: Resistance determinants in Imipenem-resistant clinical *P. aeruginosa.*

Isolate ID	MIC Value (µg/ml) for IMP	Presence of ESBL Gene				Phenotypic Detection of MBL Production		
		bla_{TEM}	bla_{SHV}	bla_{OXA}	bla_{PER1}	IMP (Zone diameter; mm)	IMP+EDTA (Zone diameter; mm)	Interpretation of MBL carriages [b]
PAO1[a]	0.5	–	–	–	–	19	20	Negative
PU1	8	–	–	–	–	0	0	Negative
PU5	8	–	–	–	–	0	15	Intermediate
PU6	8	+	–	+	–	0	15	Intermediate
PUS1	8	–	–	–	–	0	15	Intermediate
PS1	16	–	–	–	–	0	17	Positive
PS2	8	–	–	–	–	0	15	Intermediate
PU17	128	–	–	–	–	0	22	Positive
PU26	8	–	–	–	–	0	20	Positive
PWS12	32	–	+	–	–	0	20	Positive
PWS17	128	–	–	–	–	0	24	Positive
PWS18	128	–	–	–	–	0	21	Positive
PWS21	128	–	–	–	–	0	24	Positive
PWS22	16	–	–	–	–	0	22	Positive
PWS23	16	–	–	–	–	0	16	Intermediate
PWS25	8	–	–	–	–	0	16	Intermediate
PWS26	128	–	–	–	–	0	23	Positive

Notes: Here, '+' denotes the presence of the ESBL gene; '−' indicates the absence of the ESBL gene; IMP= Imipenem; [a]PAO1: Reference strain in susceptibility testing and β-lactamase assays (NCBI: txid208964), [b] interpretation whether the bacterial β-lactamase enzymes carry metallic cofactor or not. Negatives (PAO1 and PU1) indicate that β-lactamase of the particular isolates do not have metallic cofactor; Positives indicate the strong evidence of metal carriage in their β-lactamase enzymes; intermediate indicate weak comparative evidence of metal carriage in their β-lactamase enzymes.

Table 4: Resistance Determinants in Ceftazidime-Resistant Clinical *P. aeruginosa.*

Isolate ID	MIC Value (µg/ml) for CAZ	Presence of ESBL				Presence of AmpC	Phenotypic test for AmpC overproduction		Presence of OprD
		bla_{TEM}	bla_{SHV}	bla_{OXA}	bla_{PER1}		Observation	Interpretation	
PU17	128	–	–	–	–	+	Indentation	Positive Overproducer	+
PWS5	32	–	–	–	–	+	Flattening	Intermediate Overproducer	+
PWS12	128	–	+	–	–	+	Indentation	Positive Overproducer	+
PWS17	128	–	–	–	–	+	Indentation	Positive Overproducer	–
PWS18	128	–	–	–	–	+	Indentation	Positive Overproducer	+
PWS22	128	–	–	–	–	+	Indentation	Positive Overproducer	+
PWS23	128	–	–	–	–	+	Indentation	Positive Overproducer	+

Here, '+' denotes the presence of the *bla*/OprD gene; '−' means the absence of the *bla*/OprD gene; CAZ= Ceftazidime; [a] Negative PCR amplification of OprD indicating possible mutational loss of the gene.

analyzed for relative quantification of OprD gene expression by one-step qPCR. All isolates from different resistance patterns displayed decreased expression of OprD, and 8 of the 14 representative isolates showed relative expression of ≤10% compared to that of *P. aeruginosa* susceptible control PAO1 (Table 5). Two isolates with XDR phenotypes (PWS22, PWS18; Table 5) identified a much-reduced expression level of OprD (≤5% in comparison to susceptible control PAO1). Expression level analysis further revealed imipenem-resistant isolates demonstrated as low as ≤20% of OprD expression.

Table 5: Association Between Multidrug Resistance and OprD Expression Level.

Sample ID	Antibiograms Profile										mRNA Expression for OprD[a]
	AMP	CRO	CTX	CAZ	IPM	CIP	LEV	CN	AZM	TET	
PAO1	-	-	-	-	-	-	-	-	-	-	1.0
PU21	+	+	+	-	-	-	-	-	-	+	0.116629
PU19	+	+	+	-	-	-	-	-	+	+	0.233258
PUS1	+	+	+	-	+	-	-	-	+	-	0.189465
PWS5	+	+	+	+	-	-	-	-	+	+	0.307786
PWS9	+	+	+	-	-	+	+	-	+	-	0.012691
PU22	+	+	+	-	-	+	+	-	-	+	0.009618
PU6	+	+	+	-	+	-	-	+	+	+	0.108819
PU20	+	+	+	-	-	+	+	-	+	+	0.054409
PU5	+	+	+	-	+	+	+	+	+	-	0.189465
PWS1	+	+	+	-	-	+	+	+	+	+	0.267943
PWS21	+	+	+	-	+	+	+	+	-	+	0.050766
PWS22	+	+	+	+	+	+	+	+	-	+	0.011842
PWS18	+	+	+	+	+	+	+	+	+	+	0.041235

Notes: Here, AMP=Ampicillin; CRO=Ceftriaxone; CTX=Cefotaxime; CAZ=Ceftazidime; IPM= Imipenem; CIP=Ciprofloxacin; LEV=Levofloxacin; CN=Gentamycin; TET=Tetracycline; AZM=Azithromycin; '+'= Resistant; '- '= Susceptible. PAO1= Reference Strain, [a] Relative value of OprD expression, where 1.0 is equivalent to the most optimum expression by the reference strain PAO1.

Catheter swab isolates showed a higher risk of resistance than urine-origin *P. aeruginosa* (OR=3.01; 95% CI=1.88, 5.04, p=0.032) in the ceftriaxone (CTX) assessment. Imipenem (IMP) and ciprofloxacin (CIP) showed a similar higher significant risk of resistance compared to the isolates of urine specimen by 18.9 times (95%= 1.65, 29.4, p=0.018) and by 14.3 times (95% CI= 1.16, 23.6, p=0.015), respectively. Both gentamicin (CN) and levofloxacin (LEV) exhibited significantly higher resistance risk by 14.4 and 10.8 times and 24.5 and 20.1 times, respectively, in catheter swabs and other specimens (Table 6).

Table 6: Logistic Regression Model of Predicting Resistance in *Pseudomonas aeruginosa* in the different clinical specimens.

	Susceptible	Resistance	Resistance	
Specimens			**OR (95% CI)**	**p-value**
AMP				
Urine	0	23(100%)	-	
PUS	0	10(100%)		
Secondary wound infection swab	0	11(100%)	-	
Catheter swab	0	5(100%)		
Others$	0	9(100%)	-	
CAZ				
Urine	23(100%)	0	Ref. (1)	
PUS	10(100%)	0	-	
Secondary wound infection swab	8(72.7%)	3(27.3%)	0.79(0.68, 3.85)	0.882
Catheter swab	3(60.0%)	2(40.0%)	1.65(0.78, 4.25)	0.525
Others$	5(55.6%)	4(44.4%)	0.99(0.35, 4.00)	0.999
CRO				
Urine	0	23(100%)	-	
PUS	0	10(100%)	-	
Secondary wound infection swab	0	11(100%)		
Catheter swab	0	5(100%)	-	
Others$	0	9(100%)	-	
CTX				
Urine	0	23(100%)	Ref. (1)	
PUS	1	9(90.0%)	0.98(0.88, 3.28)	0.788
Secondary wound infection swab	0	11(100%)	-	
Catheter swab	1(20.0%)	4(80.0%)	3.01(1.88, 5.04)	0.032
Others$	1(11.1%)	8(88.9%)	0.99(0.17, 7.66)	0.887
IMP				
Urine	19(82.6%)	4(12.1%)	Ref. (1)	
PUS	10(100%)	0	-	
Secondary wound infection swab	6(54.6%)	5(45.5%)	3.97(0.79, 19.7)	0.093
Catheter swab	1(20.0%)	4(80.0%)	18.9(1.65, 29.4)	0.018
Others$	1(25.0%)	3(75.0%)	14.3(1.16, 23.6)	0.038
CIP				
Urine	19(82.6%)	4(17.4%)	Ref. (1)	
PUS	10(100.0%)	0	-	
Secondary wound infection swab	7(63.6%)	4(36.4%)	2.69(0.53, 13.9)	0.231
Catheter swab	1(20.0%)	4(80.0%)	18.9(1.65, 29.4)	0.018
Others$	1(25.0%)	3(75.0%)	14.3(1.16, 23.6)	0.038
LEV				
Urine	18(78.3%)	5(21.7%)	Ref. (1)	
PUS	10(100%)	0	-	
Secondary wound infection swab	7(63.6%)	4(36.4%)	2.05(0.42, 9.97)	0.370
Catheter swab	1(20.0%)	4(80.0%)	14.4(1.30, 21.5)	0.018

	Susceptible	Resistance	Resistance	
Others$	1(25.0%)	3(75.0%)	10.8(1.09, 17.3)	0.049
CN				
Urine	20(87.0%)	3(13.0%)	Ref. (1)	
PUS	10(100%)	0	-	
Secondary wound infection swab	7(63.6%)	4(36.4%)	2.45(0.42, 9.97)	0.570
Catheter swab	1(20.0%)	4(80.0%)	24.5(3.32, 18.2)	0.004
Others$	1(25.0%)	3(75.0%)	20.1(2.34, 19.9)	0.009
AZM				
Urine	4(17.4%)	19(82.6%)	Ref. (1)	
PUS	4(40.0%)	6(60.0%)	3.16(0.60, 16.6)	0.174
Secondary wound infection swab	4(36.4%)	7(63.6%)	2.69(0.53, 13.9)	0.231
Catheter swab	1(20.0%)	4(80.0%)	1.19(0.10, 13.6)	0.890
Others$	2(50.0%)	2(50.0%)	5.26(0.51, 16.3)	0.172
TET				
Urine	2(8.70%)	21(91.3%)	Ref. (1)	
PUS	1(10.0%)	9(90.0%)	1.16(0.09, 14.6)	0.905
Secondary wound infection swab	2(18.2%)	9(81.8%)	2.23(0.32, 15.5)	0.419
Catheter swab	1(20.0%)	4(80.0%)	2.51(0.21, 20.3)	0.471
Others$	0	4(100%)	4.48(0.97, 14.4)	0.078

Others$ Tracheal aspirate, blood, and burn wounds.

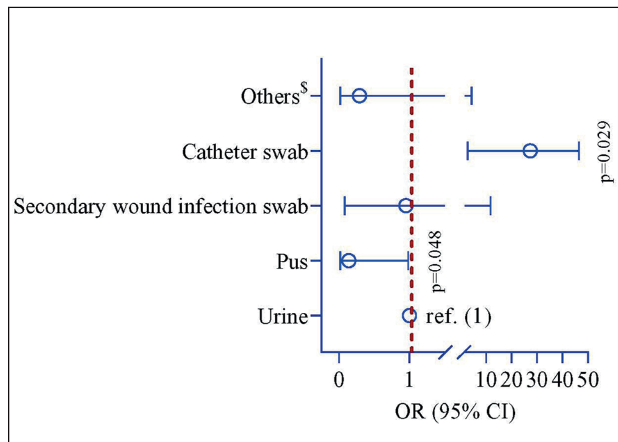

Figure 6: Risk of multidrug resistance (MDR) in specimens (secondary wound infection swab, catheter swab, and others) compared to samples from urine. Logistic regression was used to estimate the p-value. OR, odds ratio, others $ Tracheal aspirate, blood, and burn wound.

The logistic regression model was used to estimate the p-value. OR, Odds ratios were measured resistance compared to susceptibility.

The odds of getting multidrug resistance were significantly lower in the pus specimens (OR=0.14; 95% CI=0.02, 0.98, p=0.048). In comparision, catheter swabs had considerably higher odds of multidrug resistance by 27.4 times (95% CI 2.74, 46.5, p=0.029) compared to samples from urine (Figure 6).

Discussion

The resistance of *P. aeruginosa* is multifactorial, depending on an intricate interplay of intrinsic and acquired resistance determinants [11]. Several studies have been reported from Bangladesh on the antimicrobial susceptibility and resistance of *P. aeruginosa* [49, 64-66]. Still, not much has been explored regarding MDR and the intrinsic factors of antimicrobial resistance in clinical *P. aeruginosa*. The present research was done to detect the occurrence of MDR *P. aeruginosa*, investigate the presence of selected virulence factors, and determine intrinsic and acquired resistance determinants of β-lactam antibiotics (penicillin, cephalosporin, and carbapenem) amongst the multidrug-resistant *P. aeruginosa* strains isolated from different clinical specimen.

This result indicates that a significant proportion (22%) of the clinical samples tested in this study were positive for *P. aeruginosa*, a common bacterial

pathogen that can cause human infections. The study also found that secondary wound swabs had the highest proportion (52%) of *P. aeruginosa* isolation, followed by urinary tract catheters (42%). Magdy *et al.,* in their study, reported a 21% prevalence of *P. aeruginosa* from the environmental samples and a higher (35%) prevalence from the clinical sample, like sputum [67]. Adedeji *et al.,* in their study conducted in Africa on various clinical samples, observed a higher prevalence (50%) of *P. aeruginosa* in ear infections [68]. A study conducted by Mahaseth *et al.,* reported a prevalence of 11.6% in the clinical samples [69]. The study by Maharjan *et al.,* conducted on 1049 clinical samples showing growth reported a lower prevalence of *P. aeruginosa* in the clinical samples [51]. One Korean study revealed that *Pseudomonas*-derived cephalosporinase (PDC) was thoroughly resistant to ceftazidime (MIC50=256 µg/ml) and cefepime (MIC50=256 µg/ml). Among entire PDC variants, 25 isolates possess MBL genes and exhibit top-level cephalosporin and carbapenem resistance. While in contrast, 36 isolates that did not foster MBL genes give away comparably lower-ranking resistance (ceftazidime, $p<0.001$; cefepime, $p<0.001$; imipenem, $p=0.003$; meropenem, $p < 0.001$) [70]. A similar study conducted in Nepal by Shidiki *et al*., [71] (4.5%) in 2019 and Shrestha *et al*., (5.1%) in 2016 reported a lower prevalence of *P. aeruginosa* in the clinical samples [72]. Shrestha *et al*., reported that the antimicrobial responsiveness pattern of pathogenic microbes like *P. aeruginosa* in the hospital set-up must regularly keep an eye on, and the susceptibility results should be decimated among all health professionals to maximize the possibility of prudent and appropriate prescribing [72].

PCR detection of the virulence factor Exotoxin A (*tox*A), Elastase B (*las*B), and Phospholipase C (*plc*H) revealed the frequent occurrence of *las*B gene among 96.2% isolates, and *plc*H and *tox*A genes were carried in 94.3% and 92.4% isolates, respectively. Furthermore, most isolates (92.5%) were found to carry all three virulence factors. This is significant as multiple virulence factors can increase the pathogenicity of bacteria and their ability to cause disease. The high prevalence of significant virulence factors confirms the clinical nature of the isolates of *P. aeruginosa* [73, 74]. A previous study in Nepal shows 95.4% of the clinical *P. aeruginosa* isolates harbor the *toxA* gene [75], which supports our study's observance. As observed in this study, the high prevalence of *las*B [76] and *plc*H [77] in clinical isolates of *P. aeruginosa* also agrees with previous reports.

P. aeruginosa were most frequently detected from secondary wound swabs in the current study. Our findings were like earlier studies [78, 79.] The present study also noticed that *P. aeruginosa* isolates carry three virulence genes, e.g., *tox*A, *las*B, *plc*H. Similar findings were reported in preceding research studies [80, 81]. Most *P. aeruginosa* isolates in our study were highly resistant to cefotaxime [82], azithromycin [83], and tetracyclines [84]. Mentioned references [82-84] reported similar statements. Khan *et al.,* in their research, reported that the majority of the cephalosporins like cefuroxime (100%), cefixime (100%), ceftriaxone (86.6 %), and cefepime (76.6%) were resistant [85].

Magdy *et al.,* in their study, reported similar higher resistance rates with cefuroxime (95.3%), cephalothin (95%), cefoxitin (95.3%), and ceftriaxone (78%). In contrast, a low resistance was observed with cefepime (15.6%), ceftazidime (19.5%), 7.8% to amikacin, and 3.1% to colistin [67]. Another study conducted by Koirala *et al*., reported imipenem to be the most effective against *P. aeruginosa* strains with a sensitivity rate of 85.1%, followed by colistin (71.3%), amikacin (64.1%) and gentamicin (56.4%). On the other hand, the highest resistance rates were observed for ceftriaxone (70.3%), chloramphenicol (65.6%), ciprofloxacin (53.3%), and ofloxacin (52.8%) against *P., aeruginosa* [86].

This study found that the lowest resistance was observed with ceftazidime. Similar observations were noticed by earlier research [87]. Nonetheless, another study revealed minimum ceftazidime-avibactam for treating MDR/XDR *P. aeruginosa* infections [88]. Several other studies reported a higher resistance figure with ceftazidime, contrary to ours. A study by Maharjan [51] reported about 53% resistance with ceftazidime, and similar figures (49%) were also reported by Mahaseth *et al.,* [69] whereas Shidiki *et al.,* [71] (90%) reported a comparatively very higher figure of resistance with ceftazidime. However, current analysis, isolates of *P. aeruginosa* were moderately susceptible to imipenem, ciprofloxacin, levofloxacin, and gentamycin. Nevertheless, another study reported that *P. aeruginosa* and MDR *P. aeruginosa* resist almost all routinely used antimicrobial agents except colistin [89]. Maharjan *et al.,* in their research, reported a similar result where the sensitivity of imipenem (90%), ciprofloxacin (65%), and gentamycin (61%) against *P. aeruginosa* was fair as compared to other antimicrobials [51]. Similar figures were also reported by Khan *et al.,* with 40% resistance observed with imipenem, 30% with gentamicin, and 26.6% with

ciprofloxacin [85]. Shrestha *et al.*, reported 36.7% resistance with ciprofloxacin, 28.8% with ofloxacin, 31% with gentamycin, and only 6.5% with Imipenem [72]. Magdy *et al.*, reported a 21% resistance with gentamicin but a higher (41%) resistance with imipenem [67].

The result indicates a high prevalence of MDR isolates of *P. aeruginosa*. Approximately 73% were found to be MDR, meaning they were resistant to three or more groups of antibiotics. This suggests that the isolates may have acquired resistance mechanisms that make them more challenging to treat with conventional antibiotics. Additionally, 12% were extensively drug-resistant (XDR), meaning they were resistant to even more classes of antibiotics (Figure 7). This is a concerning finding, as it indicates that these isolates may be almost impossible to treat with current antibiotics, leaving patients vulnerable to severe infections. A similar high prevalence of MDR *P. aeruginosa* was also reported by studies conducted by Sujakhu *et al.,* [90] (63.3%) and Koirala *et al.*, (69.1%) [86].

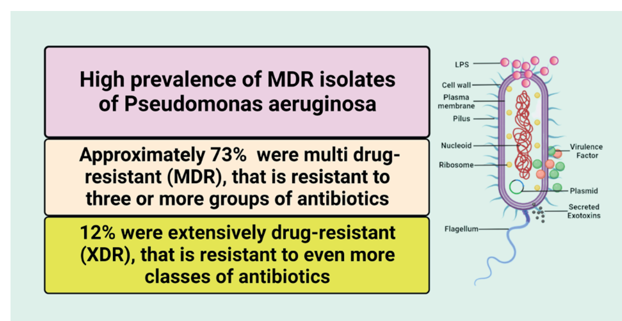

Figure 7: Diagram showing the *P. aeruginosa* isolates with a high MDR prevalence. This figure has been drawn utilizing the premium version of BioRender with the License number UP2569Q54I. **Image Credit**: Susmita Sinha.

Ribeiro *et al.,* in their study on the specimens from the intensive care unit, reported a prevalence of 48.7% for MDR *P. aeruginosa* [91]. A study conducted by Gill *et al.*, on 1915 patient sample reported that the prevalence of MDR *P. aeruginosa* was 50% and XDR *P. aeruginosa* was 2.3%, both of which were lower than our study [92]. These high rates of prevalence of MDR and XDR strains are quite concerning. Therefore, prescribing antibiotics to patients should be based on the antibiogram results to prevent the emergence of MDR or XDR strains. Compared to the previous studies, a study conducted by Hosseininassab *et al.*, in burn patients reported very high figures of resistance by *P. aeruginosa*. The prevalence of MDR strains was 95.8%, whereas XDR strains showed a high prevalence of 87.5% [93]. Mahaseth *et al.,* [69,] in their study, reported about 36.86% of isolates of *P. aeruginosa* were found to be MDR, and similar results were reported by Maharjan *et al.*, (31%) [51].

The study also identified the loss of outer membrane protein (OprD) porin protein as a factor associated with MDR status. OprD porin is known to play a crucial role in the entry of carbapenems, an important class of antibiotics, into the bacterial cell [9, 94]. Loss of OprD porin protein makes the bacteria more resistant to carbapenems, increasing their MDR status [95].

The results of this study show that only a small proportion (6%) of the resistant isolates examined carried ESBL genes, specifically bla_{TEM}, bla_{OXA}, and bla_{SHV}. This suggests that the high level of resistance observed in these isolates is not solely due to ESBL genes [96]. In 2017, Murugan *et al.*, conducted a study in India which revealed that *P. aeruginosa* bacteria contained the following β-lactamase traits: bla_{CTXM} (7%), bla_{GES1} (11%), bla_{OXA10} (33.5%), bla_{VEB} (11.5%), and bla_{TEM} (72.5%) [97]. Contrary to our result, a study by Peymani *et al.,* [98] reported that approximately 28.6% of the *P. aeruginosa* isolates were ESBL producers with the most common gene as bla_{TEM-1} (26.7%), followed by bla_{CTXM15} (17.3%), bla_{SHV1} (6.7%), and bla_{SHV12} (4%).

One more study conducted by Pakbaten *et al.*, reported that about 88% of isolates of *P. aeruginosa* were MDR in nature, and 36% were positive for each or in combination for bla_{OXA-10}, bla_{TEM} and bla_{SHV} genes, respectively [99]. A similar study conducted by Nasser *et al.*, on patients with burn and wound infections reported that about 66.3% of the isolates were MDR. The prevalence of ESBL genes was bla_{VEB} (43%), bla_{GES} (43%), bla_{CTXM} (30.7%), bla_{OXA10} (30.7%), bla_{TEM} (24.6%) and bla_{SHV} (12.3%) respectively [100]. In 2016, Al-Agamy *et al.*, conducted a study in Saudi Arabia, finding that the percentages of $bla_{CTXM,}$ bla_{PER1}, bla_{OXA10}, and bla_{VEB} were 11%, 14%, and 16%, respectively [101]. Notably, a study from Egypt by Hassuna *et al.*, in 2015 detected only 12% bla_{TEM} [102]. However, one more study result differed from earlier findings in the Middle East and other countries [103]. In contrast, another study by El-Shouny *et al.*, in 2018 found that only 60.7% of the bacteria contained bla_{OXA10} [104].

ESβL genes confer resistance to certain antibiotics, particularly β-lactam antibiotics such as penicillins, cephalosporins, and carbapenems [105]. However,

resistance to other classes of antibiotics can also occur through various mechanisms, such as mutations in bacterial genes or the acquisition of different resistance genes [28, 29, 106, 107]. Therefore, these findings suggest that the high level of resistance observed in the non-ESBL isolates may be due to alternative resistance mechanisms [11, 108, 109] which may be related to the specific antibiotics used in the study area. This highlights the importance of identifying the precise resistance mechanisms in bacterial isolates, as this information can guide the appropriate selection of antibiotics for treatment.

The result indicates a high likelihood that the imipenem resistance in these 16 *P. aeruginosa* isolates is due to the production of carbapenemase enzymes. This is because 15 of the 16 isolates were identified as carbapenemase producers, suggesting that carbapenemase genes are a major contributor to the resistance to imipenem.

In their study conducted in East India in 2019, Verma *et al*., reported a high prevalence of carbapenemase production in *P. aeruginosa* isolates [110]. The carbapenem-hydrolyzing carbapenemase-producing *P. aeruginosa* has been isolated worldwide and is a matter of concern [111]. The Centers for Disease Control and Prevention (CDC) constantly tracks the Carbapenem-Resistant *P. aeruginosa* through data from the Antibiotic Resistance Laboratory Network and CDC laboratories to identify the carbapenemase and control the spread of antimicrobial resistance [112].

P. aeruginosa is a Gram-negative bacterium frequently associated with healthcare-associated infections, including pneumonia, urinary tract infections, and bloodstream infections. Carbapenems are one of the last resort antibiotics used to treat infections caused by multidrug-resistant *P. aeruginosa*. However, the emergence of carbapenem-resistant strains of *P. aeruginosa* has become a significant public health concern (Figure 8).

The finding that 15 out of 16 imipenem-resistant *P. aeruginosa* isolates are carbapenemase producers indicates that these strains are likely highly resistant to carbapenem antibiotics. This has important implications for managing *P. aeruginosa* infections, as it suggests that alternative treatment options may need to be considered. Furthermore, the emergence of carbapenem-resistant strains of *P. aeruginosa* highlights the importance of infection control measures, including hand hygiene, surveillance, and appropriate use of antibiotics, to prevent the spread of these highly resistant bacteria. Moreover, as observed in this study, the increasing number of MDR and XDR isolates poses a greater danger for treating nosocomial infections, demanding a better understanding to prevent therapeutic failure and guide a more effective antimicrobial treatment regimen against the bacteria. This study affirms the rise of multidrug-resistant and extensively drug-resistant *P. aeruginosa* as an imminent threat in hospital settings in Bangladesh.

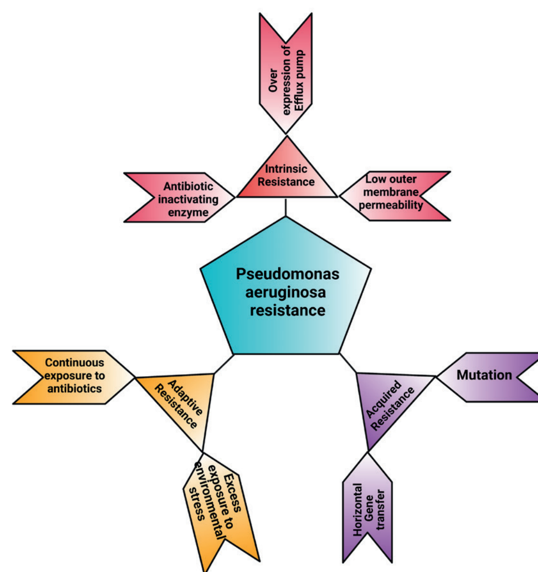

Figure 8: Diagram showing possible cause for *P. aeruginosa* antibiotic resistance. This figure has been drawn utilizing the premium version of BioRender with the License number YT2569XZB9. **Image Credit**: Susmita Sinha.

Overall, the result highlights the importance of continued surveillance and monitoring of antibiotic resistance in bacterial pathogens like *P. aeruginosa.* The study also underscores the need to develop new antibiotics and alternative treatment options to combat the growing problem of antibiotic resistance.

Conclusions

The investigation elucidates the emergence of multidrug-resistant and extensively drug-resistant *P. aeruginosa* in our clinical settings. In Bangladesh, study on the emergence of MDR and XDR *P. aeruginosa* is scarce. Still, this opportunistic pathogen is quickly evolving into one of the significant threats in our healthcare settings. Considering the diversity of armory possessed by the pathogen against clinically significant β-lactam antibiotics, as observed in this study, more attention is needed to understand the

molecular determinants to preclude the therapeutic challenge it poses.

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Conflict of Interest Statement

The authors declare no conflict of interest.

Data Availability

The data is available Principal Author only for research purposes.

Authors' Contribution

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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